EXHIBIT I

ONYX
PHARMACEUTICALS

August 11, 1995

Nelson A. Wivel, M.D.
Director, Office of Recombinant DNA Activities
National Institutes of Health, MSC 7052
6000 Executive Boulevard, Suite 302
Bethesda, MD 20892-7052

Dear Dr. Wivel:

Onyx Pharmaceuticals is developing an attenuated adenovirus (ONYX-015) for use as a cancer therapeutic to treat tumors which are defective in p53 tumor suppressor function. ONYX-015 is a human adenovirus deletion mutant which carries no exogenous genes, but which displays selective replication in tumor cells compared to normal cells due to the nature of the viral gene which is deleted. In many respects, ONYX-015 resembles attenuated live viral vaccine strains which also have restricted replication, and are therefore non-virulent.

Since this attenuated virus contains no exogenous genes, it is our belief that clinical protocols utilizing this agent are not appropriate candidates for RAC review. This letter is intended to provide you with some background information on the company and ONYX-015, and to describe the reasoning which led to our conclusion concerning RAC review. Hopefully, this information will prove sufficient for you to provide us with clear guidance on RAC review.

Onyx Pharmaceuticals was founded in 1992 by researchers from Chiron Corporation. The company's research focuses on molecular mechanisms of signal transduction by the *ras* superfamily of proteins, on cell cycle regulation, and on tumor suppressor gene function. The medical focus is on potential opportunities for therapeutic intervention in cancer, inflammation and other diseases associated with abnormal cell growth and behavior.

ONYX-015 is an attenuated adenovirus serotype 2 which selectively targets cancer cells which do not have a functional p53 gene product. The virus replicates in and kills tumor cells deficient in p53 tumor suppressor activity (p53-), and spreads to other cells with the p53- phenotype. The virus does not replicate to a significant extent in cells with normal p53 function (p53+). The selectivity of ONYX-015 is conferred through deletion of part of the viral genome within the E1B region, which results in the virus being unable to

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produce the E1B 55 kD protein. The function of this protein in wild-type adenovirus is to bind and inactivate p53, thus allowing the virus to replicate. Since ONYX-015 cannot produce the E1B 55 kD protein and thus cannot inactivate p53, it cannot efficiently reproduce in normal cells with functional p53. However, in tumor cells which lack functional p53, it actively reproduces and induces cell lysis.

ONYX-015 is a plaque purified isolate of a virus constructed in the laboratory of Dr. Arnold Berk (D. Barker and A. Berk, Virology *156:* 107-121, 1987) through modification of a Group C human adenovirus, serotype 2. The attenuated strain contains a deletion in the E1B 55 kD region between nucleotides 2496 and 3323, and the insertion of a small linker at this position which generates a stop codon at nucleotide 3336. In order to eliminate expression of a truncated 55 kD protein, the attenuated strain also contains a C to T transition at position 2022 in E1B, which generates another stop codon three amino acids downstream from the translational initiation codon of this protein. The result of these manipulations is a virus which is altered only in its ability to express the E1B 55 kD protein.

A series of *in vitro* studies have shown that ONYX-015 is cytopathic for a wide range of p53- tumor cell lines, but not for p53+ tumor cell lines or normal cell lines. Wild-type adenovirus is cytopathic in both p53- and p53+ cell lines. Quantitation of viral yields produced by p53- or p53+ cells during a single round of infection indicates that wild-type virus yield is independent of the host cell's p53 status, while ONYX-015 produces a significant burst of progeny virus only in p53- host cells. In the permissive p53- cells, the yields from attenuated and wild-type virus are comparable.

In vivo studies in human tumor xenografts in nude mice show that ONYX-015 treatment produces viral replication and tumor cell lysis leading to tumor necrosis and regression in p53- xenografts, but not in p53+ xenografts. Complete, durable regressions of p53- tumors were obtained in a significant number of cases. No ONYX-015-related toxicity was observed in safety studies performed in cotton rats, a species which is permissive for human adenovirus replication.

Our conclusion that clinical protocols utilizing ONYX-015 are not appropriate candidates for RAC review rests on both the nature of the virus and a careful review of the NIH Guidelines for Research Involving Recombinant DNA Molecules as noted below.

1. Adenovirus agents per se are not subject to RAC review, but only adenovirus agents which contain exogenous genes and are thus considered "recombinant" as defined in the NIH Guidelines.

2. Section I-B of the NIH Guidelines defines recombinant DNA molecules as follows: "In the context of the *NIH guidelines*, recombinant DNA molecules are defined as either: (i) molecules that are constructed outside living cells by joining natural or

synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above." The succeeding paragraph of Section I-B, contains this additional statement: "If the synthetic DNA segment is not expressed *in vivo* as a biologically active polynucleotide or polypeptide product, it is exempt from the *NIH Guidelines*." No natural or synthetic DNA segments were joined to the parent adenovirus to generate ONYX-015, with the exception of a small (12 bp) polylinker fragment which produces no biologically active product. The result of the manipulations used to create ONYX-015 is a virus which retains all of the wild-type adenovirus genes, with the exception of the deleted E1B 55 kD gene, and no exogenous genes. Since no natural or synthetic gene segments were joined to the wild-type adenovirus sequences, we believe that ONYX-015 falls outside the range of molecules covered by the NIH guidelines, and, therefore, outside the range of agents reviewed by RAC.

3. Section III-E-2 describes the following experiments as exempt from the NIH Guidelines: "Those that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent." All ONYX-015 sequences, with the exception of the inactive 12 bp polylinker segment, are derived from human adenovirus type 2 and appear, therefore, to be exempt from the Guidelines.

4. Appendix M of the NIH Guidelines states that: "...research proposals involving the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into human subjects (human gene transfer) will be considered through a consolidated review process involving both the NIH and the FDA." Since ONYX-015 does not fall within the definition of "recombinant DNA" presented in the Guidelines, it appears to be exempt from RAC review. Onyx is in the process of preparing an Investigational New Drug Application for an ONYX-015 clinical trial which will be presented to the FDA for their review.

The points summarized above have led Onyx to the conclusion that RAC review of clinical protocols utilizing ONYX-015 is not appropriate. However, we would be pleased to meet with you to discuss this issue further. Onyx is committed to maintaining open communication with ORDA on this matter.

Thank you for your careful consideration of this issue. We will contact you next week by telephone to follow-up on this matter.

Sincerely,

Christopher A. Maack

Christopher A. Maack, Ph.D.
Director, Project Management

ONYX
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September 28, 1995

Nelson A. Wivel, M.D.
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Dear Dr. Wivel:

On August 11, 1995, I wrote to you with a description of ONYX-015, the attenuated adenovirus which will be employed in Onyx's planned Phase I clinical trial in patients with head and neck cancer. While continuing our evaluation of this product, we have obtained additional information on the structure of ONYX-015. With this letter we would like to provide you with an updated description of this agent, including this new information.

As previously indicated, ONYX-015 is a clone of the human adenovirus dl1520 which was obtained from the laboratory of Dr. Arnold Berk at UCLA, where it was constructed and characterized. The primary feature of the virus is a deletion in the adenovirus serotype 2 E1B 55 kD region. This mutation blocks efficient replication of the virus in cells with normal p53 tumor suppressor activity, but does not affect viral replication in p53 deficient tumor cells. The details of this E1B 55 kD deletion were presented in my letter of August 11.

Recent discussions with Dr. Berk revealed that ONYX-015 is a chimeric virus created by homologous recombination between the modified early region of human adenovirus serotype 2 described above, and regions of a viable human adenovirus serotype 5 variant, dl309. Adenovirus serotypes 2 and 5 both belong to Group C, have extensive DNA and amino acid sequence homology (98%), and are considered essentially interchangeable by adenovirus experts. This high level of homology facilitates homologous recombination in cells co-infected with virus of both serotypes (J. Williams, T. Grodzicker, P. Sharp and J. Sambrook, Cell *4*: 113-119, 1975), or in cells co-transfected with overlapping DNA fragments from each serotype (G. Chinnadurai, S. Chinnadurai and J. Brusca, J. Virol. *32*: 623-628, 1979).

Dl1520 was generated by *in vivo* overlap recombination between complementing fragments of the genomes of adenovirus serotypes 2 and 5 in co-transfected HEK293 cells. The result is an attenuated virus which contains the E1 region of human adenovirus serotype 2, including the E1B 55 kD deletion described above, fused to the remaining downstream sequences of the adenovirus serotype 5 variant dl309, including the dl309 modifications detailed below.

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The details of dl309's structure have recently been obtained from Dr. Frank Graham (McMaster University, Hamilton, Ontario) in a preprint of a paper which is soon to be published (A. Bett, V. Krougliak and F. Graham, Virus Research, in press). Dl309 is in widespread use because of the ease with which it can be genetically manipulated. Starting with a wild-type adenovirus serotype 5 population, dl309 was isolated using a stepwise viral DNA restriction/re-ligation procedure that produced variants in which three out of the four normal Xba I restriction sites had been lost (N. Jones and T. Shenk, Cell *13*: 181-188, 1978, and N. Jones and T. Shenk, Cell *17*: 683-689, 1979). This procedure selects for spontaneously occurring viable variants missing one or more Xba I sites since fewer ligation events are required to regenerate a complete variant DNA than would be required to produce an intact wild-type viral DNA. Shenk and other investigators have confirmed that dl309 is fully viable and grows to high titers on several standard cell lines.

The Xba I sites at 29.5 and 79.5 map units (m.u.) in dl309 were lost due to deletions of two base pairs (bp) (nucleotides 10,594 and 10,595) and 6 bp (nucleotides 28,597-28, 602), respectively (A. Bett, V. Krougliak and F. Graham, Virus Research, in press). These deletions alter the expression of VAI(A) RNA and the E3 6.7 kD protein, and may lead dl309 infected cells to display increased sensitivity to interferon-induced inhibition of viral replication. Graham and co-workers also showed that the Xba I site at 84.8 m.u. was lost due to a 746 bp deletion (nucleotides 30,005-30,750) and a 642 bp insertion of what appears to be salmon DNA at this site. The inserted DNA is most likely derived from the salmon sperm DNA used as a carrier during the cellular transfections performed while isolating dl309. This insertion occurred in a region which is known to occasionally acquire non-viral DNA during virus growth. The deletion/insertion disrupts the coding sequences of the E3 10.4 kD, 14.5 kD and 14.7 kD proteins, and may increase the sensitivity of dl309 infected cells to lysis by tumor necrosis factor. The deletion/insertion is also predicted to create a fusion protein in which the last 18 amino acids in the E3 10.4 kD protein are replaced with 27 amino acids encoded by the inserted sequence. It is not known if this predicted fusion protein accumulates in dl309 infected cells. An extensive search of gene bank sequences reveals no significant homologies to this predicted 27 amino acid peptide, so it has no known biological activity if it does accumulate.

This additional structural information does not alter the previously described safety or efficacy profile of the virus outlined in my letter of August 11. Please do not hesitate to contact me by telephone (510-262-8709) or FAX (510-222-9758) if you have any questions.

Sincerely,

Christopher A. Maack

Christopher A. Maack, Ph.D.
Director, Project Management